#26
GND

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Jan E. Schnitzer and Philip Oh		
Application No.:	09/208,195	Group:	1644
Filed:	December 9, 1998	Examiner:	P. Nolan
For:	IMMUNOISOLATION OF CAVEOLAE		

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, P.O. Box 2327, Arlington, VA 22202

on 9/9/02 Christina McSweeney
Date Signature

Christina McSweeney
Typed or printed name of person signing certificate

DECLARATION UNDER 37 C.F.R. §1.131
OF JAN E. SCHNITZER, M.D.

Assistant Commissioner for Patents
P.O. Box 2327
Arlington, VA 22202

Sir:

I, Jan E. Schnitzer, of 1475 Trabert Ranch Road, Encinitas, California 92024, hereby declare and state that:

1. I am a named inventor on the above-reference patent application. I have reviewed the application and the claims as pending prior to executing the Declaration.

2. I understand that the Examiner has rejected the claims of the referenced patent application as being anticipated by Scherer, *et al.* (*JBC* 272(46):29337-29346 (1997), herein referred to as "Scherer *et al.*"), stating that Scherer *et al.* describe two monoclonal antibodies, including mAb 2234 (i.e., CAV antibody). In particular, the Examiner stated that Mab 2234 was used by Scherer *et al.* "to immunoisolate caveolae." I have reviewed Scherer *et al.* prior to executing this Declaration.

3. Immunoisolation of caveolae, as described in the referenced patent application, differs significantly from immunoprecipitation of caveolin, as described by Scherer *et al.*, in the goals of the processes, the steps used, and also in the ultimate product that is obtained. Although caveolin forms an oligomeric structural cage surrounding intact caveolae, it is only one component of many that form caveolae, and isolation of caveolin is not equivalent to isolation of an intact caveola.

4. Immunoprecipitation refers to separation, usually of a single protein from the environment in which it is found, using an antibody. Immunoprecipitation frequently uses a means of disrupting membranes (for example, detergent), to facilitate separation of the protein of interest from the environment in which it is found (for example, a cell lysate). The ultimate goal of immunoprecipitation is isolation of the individual protein of interest from other components.

5. Scherer *et al.* describe a method of immunoprecipitation of the protein, caveolin. They indicate that three different caveolin genes (Cav-1, Cav-2, and Cav-3) encoding four different subtypes of caveolin have been described, and that study of caveolin-2 has been hampered by a lack of caveolin-2-specific antibodies. They describe a mAb that recognizes caveolin-2 protein but not other known members of the caveolin gene family, and characterize expression and localization of caveolin-2 protein using that antibody. They utilize CAV (mAb 2234), which binds to caveolin-1 for immunoprecipitation. In the immunoprecipitation described by Scherer *et al.*, the cells are lysed in the presence of detergent (see "*Immunoprecipitation*" discussion), which not only disrupts but also destroys membranes, and strips lipids as well as proteins from cellular components, thereby exposing caveolin and allowing the antibody to bind to it. Thus, the methods of

Scherer *et al.* strip away both lipids and other proteins attached to caveolin, disrupting the structure of the caveolae and thereby eliminating the possibility of isolating the intact caveolae themselves.

6. In contrast to the methods described by Scherer *et al.*, the immunoisolation described in the referenced application is designed to separate a whole, complex organelle (a caveola) from plasma membranes of a cell, using an antibody. Immunoisolation of caveolae, unlike the immunoprecipitation of caveolin described above, avoids the use of detergents, because detergents may alter the composition of the membrane and thereby hinder the ability to isolate caveolae in their native state.

7. In the immunoisolation methods of the invention, a sample comprising plasma membranes is used. This is in direct contrast to Scherer *et al.*, who do not use any sample comprising plasma membranes. Plasma membranes must be present in order to perform the methods of isolating caveolae, as the caveolae are organelles that are an integral part of the membranes. The caveolae are then separated from the plasma membranes, resulting in the isolation of the caveola from the plasma membranes. Thus, it can be seen that immunoprecipitation of caveolin, as described by Scherer *et al.*, differs significantly from immunoisolation of caveolae, as described in the referenced patent application.

I further declare that all statements made herein of my knowledge are true and that all statements made on other information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Jan E. Schnitzer

9/9/02

Date